4232–4236 *Nucleic Acids Research, 2000, Vol. 28, No. 21*

Hyper-resistance of meiotic cells to radiation due to a strong expression of a single *recA*-like gene in *Caenorhabditis elegans*

Takako Takanami, Akiyuki Mori, Hideyuki Takahashi and Atsushi Higashitani*

Institute of Genetic Ecology, Tohoku University, 2-1-1 Katahira, Sendai 980-8577, Japan

Received July 14, 2000; Revised and Accepted September 18, 2000

ABSTRACT

Sensitivity of meiotic cells to DNA damaging agents is little understood. We have demonstrated that the meiotic pachytene nuclei in the *Caenorhabditis elegans* gonad are hyper-resistant to X-ray irradiation, but not to UV irradiation, whereas the early embryonic cells after fertilization and the full grown oocytes are not. The *Ce-rdh-1* gene [*RAD51*, *DMC1* (*LIM15*), homolog 1 or *Ce-rad-51*], which is essential for the meiotic recombination, is the only bacterial *recA*-like gene in the nematode genome, and is strongly expressed in the meiotic cells. Following silencing of the *Ce-rdh-1* gene by RNA interference, the meiotic cells become more sensitive to X-ray irradiation than the early embryonic cells. This is the first report that meiotic cells are hyper-resistant to DNA strand breaks due to the high level of expression of the enzyme(s) involved in meiotic homologous recombination.

INTRODUCTION

Double-strand breaks (DSBs) of DNA, caused by ionizing radiation and some chemical agents, are the most dangerous forms of damage to genetic material (1). All cells maintain repair systems for such breaks, however, in certain cells, DSBs occur normally in processes such as meiosis and the generation of diversity in the immune system in vertebrates (2,3). Little is known about the sensitivity of such cells to ionizing radiation and DNA damaging agents. The nematode *Caenorhabditis elegans* is a useful experimental organism for studies on the several processes of development and differentiation including meiosis (4–7). In the adult *C.elegans* hermaphrodite, after approximately 150 sperm are produced in each of two gonadal arms at the L4 larval stage, germ cell differentiation switches to oogenesis. The germline nuclei of the distal arm mitotically divide and thereafter progress into meiosis, from leptotene distally to diplotene of meiotic prophase I proximally. Full grown oocytes then arrest at diakinesis of meiotic prophase I, enter the spermatheca and are fertilized. After fertilization, the meiotic divisions are completed and embryogenesis ensues in the uterus. Thus, the adult hermaphrodite provides a convenient model system to study the sensitivity to DNA damaging agents of both meiotic cells and somatic cells in early embryogenesis.

Previous studies have shown that meiotic homologous recombination is initiated by DSBs caused by an enzymatic reaction during passage from the leptotene to pachytene stage in yeast and *C.elegans* (8–10). A central reaction of homologous recombination involves two *recA*-like genes, *RAD51* and *DMC1* (*LIM15*) which carry out DNA strand exchange between single-stranded and double-stranded homologous DNA segments (2,8,11–15). In the nematode *C.elegans*, we have characterized a *recA*-like gene, termed *Ce-rdh-1* [*C.elegans RAD51*, *DMC1* (*LIM15*), homolog 1 (16) and also termed *Ce-rad-51* (17)] that is essential for the meiotic process (16). In the work reported here, we examine the sensitivity of both meiotic cells and somatic cells of the nematode *C.elegans* to X-ray irradiation and its correlation to the expression of the *Ce-rdh-1* gene using an RNA interference (RNAi) method (18).

MATERIALS AND METHODS

Handling procedures for *C.elegans*

The general methods used to culture and handle *C.elegans* N2 wild-type strain have been described (4). *Ce-rdh-1* RNAi was carried out as described previously (16,18). For X-ray irradiation four young gravid hermaphrodites and *Ce-rdh-1* RNAi hermaphrodites 24 h after injection (I0) were irradiated with X-rays (1.6 Gy/min; Hitachi Co., LTD model MBR1520R). Following irradiation, the animals were immediately transferred to new culture plates and survival of their eggs (F1 generation) was measured. UV irradiation was carried out by a UV transilluminator, and its UV (254 nm) dose was measured by a UVX digital radiometer (UVP Inc.).

Expression analyses of the *Ce-rdh-1* gene

In situ hybridization to whole-mount animals and to dissected gonads were carried out by the methods described (19,20). Sense (control) and antisense DNA probes of the *Ce-rdh-1* gene were prepared with digoxigenin-labeled dUTP and *Taq* polymerase (Boehringer Mannheim). RT–PCR reactions were carried out with 5′-TGC CAC TTT TCG ACC CGA AC and 5′-GAT TGA GTA GGT CGC TTC GG, and 5′-CGT GGT TAC TCT TTC ACC ACC ACC GCT G and 5′-CAT TTA GAA GCA CTT GCG GTG AAC GAT GG as primers for

*To whom correspondence should be addressed. Tel: +81 22 217 5715; Fax: +81 22 723 8218; Email: ahigashi@ige.tohoku.ac.jp

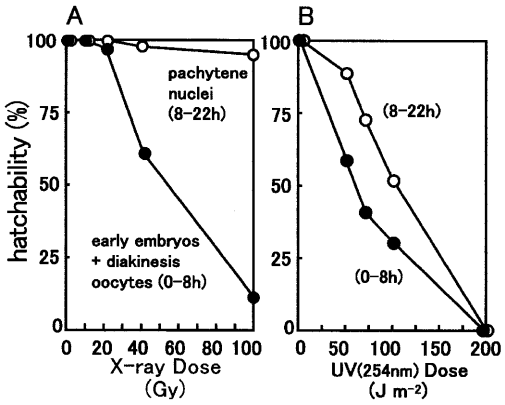

Figure 1. Survival of the eggs laid by wild-type *C.elegans* hermaphrodites following X-ray irradiation and UV irradiation. Four young gravid hermaphrodites (N2 wild-type) were irradiated with 10–100 Gy of X-rays (**A**) and 50–200 J m^{-2} of UV (254 nm) irradiation (**B**). The eggs laid during 0–8 h following irradiation (average 36 eggs laid per animal: irradiated at early embryogenesis and diakinesis) and 8–22 h (average 70 eggs laid: irradiated at pachytene) were collected separately and their hatching rate was scored as a survival rate.

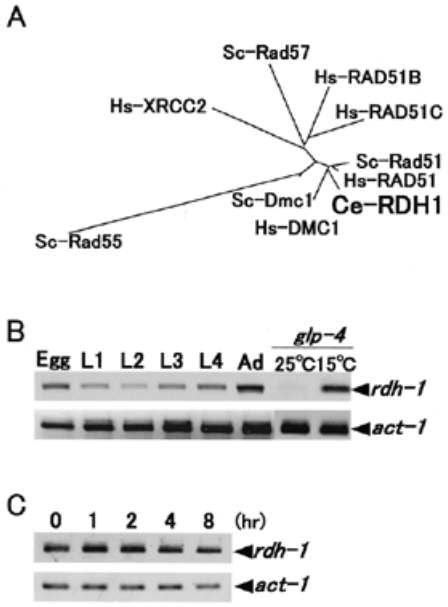

Figure 2. Characterization of a *C.elegans recA*-like gene *Ce-rdh-1*. (**A**) Comparison of the putative gene product of *Ce-rdh-1* with eukaryotic *recA*-like genes in yeast and human. The phylogenetic tree was produced using the full-length sequences: Sc-Rad51 (*Saccharomyces cerevisiae*:DNA database accession no. D10023); Hs-RAD51 (human: D14134); Ce-RDH1 (*C.elegans*: AB011382); Hs-DMC1 (D64108); Sc-Dmc1 (U18922); Sc-Rad55 (D10481); Hs-XRCC2 (Y08837); Sc-Rad57 (M65061); Hs-RAD51B (U84138); Hs-RAD51C (AF029669). (**B**) RT–PCR analysis of *Ce-rdh-1* gene expression during developmental process. Total RNA was isolated from the eggs at early embryogenesis (lane 1), the L1 larvae to wild-type young adult hermaphrodites (lanes 2–6), and *glp-4* mutant adults cultured at either a non-permissive temperature (lane 7, 25°C) or a permissive temperature (lane 8, 15°C). (**C**) Effects of X-ray irradiation on the *Ce-rdh-1* gene expression. The expression levels of *Ce-rdh-1* mRNA in the adult hermaphrodites were detected by RT–PCR at the indicated periods after irradiation with 40 Gy of X-rays [lanes 1–5: time 0 (no irradiation control), 1, 2, 4 and 8 h after]. RT–PCR was performed with primers specific for *Ce-rdh-1* and *act-1* (actin gene). DNA fragments were run on a 1.5% agarose gel, stained with ethidium bromide and quantified by densitometric scanning of Polaroid 665 negative film. Arrowheads indicate the positions of the *Ce-rdh-1* and *act-1* RT–PCR fragments.

specific amplification of *Ce-rdh-1* and *act-1* mRNA (~500 nt), respectively, using a one step RT–PCR kit (Gibco BRL). As the template for RT–PCR, total RNA (0.1 μg) was extracted from the eggs at early embryogenesis, the L1 larvae to wild-type young adult hermaphrodites, and *glp-4* mutant adults cultured at either a non-permissive (25°C) or permissive temperature (15°C) with TRIzol Reagent (Gibco BRL). Cycling conditions were 50°C for 30 min (reverse transcription), 94°C for 2 min, 28–35 cycles of 94°C for 30 s, 55°C for 30 s and 72°C for 1 min, then 72°C for 10 min.

RESULTS AND DISCUSSION

Hyper-resistance of meiotic cells (pachytene nuclei) to X-ray irradiation

Survival of eggs from a young gravid hermaphrodite after X-ray irradiation was measured and the results are shown in Figure 1A. The hatching (survival) rate of the eggs laid during 0–8 h after irradiation with 40 or 100 Gy of X-rays was ~61 and 12%, respectively. The hatching rate during 8–22 h did not decrease by more than 5% at 100 Gy. Following irradiation, one young gravid hermaphrodite had about 15 fertilized eggs and 15 full grown oocytes, and more than 100 pachytene nuclei in the gonad. The eggs laid between 0 and 8 h had been irradiated at the stage of embryogenesis and at the diakinesis stage in full grown oocytes while those eggs laid between 8 and 22 h had been irradiated at the pachytene stage of meiotic division I. In contrast, the resistance to UV irradiation in the meiotic pachytene nuclei was slightly higher than that in the early embryos and the full grown oocytes (Fig. 1B). These results show that the pachytene nuclei are specifically hyper-resistant to X-ray irradiation. At least two possible mechanisms can explain this phenomenon: a conformation of the chromosomes that is resistant to DSBs damage (e.g. paired structure with synaptonemal complex) and/or the presence of an efficient DSB repair system.

A single *recA*-like gene, *Ce-rdh-1* in *C.elegans*

We have previously characterized a *C.elegans recA*-like gene, termed *Ce-rdh-1*, that is essential to the meiotic process (16). From a BLAST search analysis of the complete genome of *C.elegans*, we could not find any additional *recA*-like genes and no homologs of *RAD52*, *RAD55* and *RAD57* which interact with *RAD51*, although these genes are widely conserved in yeast and vertebrates (21; Fig. 2A).

We studied the expression of the *Ce-rdh-1* gene by *in situ* hybridization and RT–PCR reaction. The *Ce-rdh-1* mRNA was detected in the eggs at early embryogenesis by RT–PCR analysis, however, the levels were significantly reduced in the L1 and L2 larvae and in the *glp-4* mutant adults (22) which have a defect of germ line proliferation (Fig. 2B). The CeRDH1 protein was detected using anti-CeRDH1 antibodies and found to be strongly expressed in the wild-type adults and eggs but not in the L2 larvae or the *glp-4* mutants which reflected its mRNA expression pattern (data not shown). Moreover, the expression levels of *Ce-rdh-1* mRNA in the adult

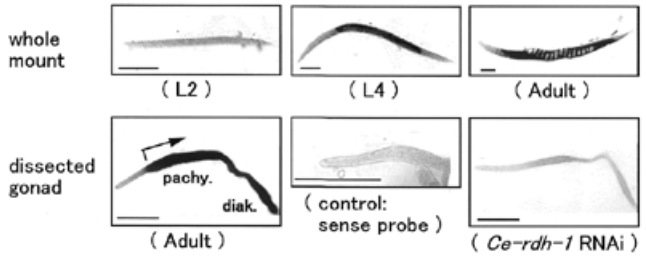

Figure 3. Expression analysis of the *Ce-rdh-1* gene by *in situ* hybridization. *In situ* hybridization of the *Ce-rdh-1* gene to whole-mount animals and to dissected gonads were carried out. The *Ce-rdh-1* gene was strongly expressed in the gonad after the L4 larval stage and in the region of meiotic cells (indicated by an arrow) during pachytene to diakinesis. As a control, the sense probe of the *Ce-rdh-1* gene was used in the hybridization (control). In the dissected gonad from a parent injected with dsRNA of *Ce-rdh-1* 24 h after injection, the strong signal disappeared (*Ce-rdh-1* RNAi). Scale bars represent 0.1 mm.

hermaphrodites increased ~2-fold during 1–2 h after irradiation with 40 Gy of X-rays (Fig. 2C). The results of *in situ* hybridization of larvae and adult hermaphrodites indicated that the gene was strongly and specifically expressed in the meiotic pachytene to diakinesis cells of the gonad (Fig. 3).

X-ray sensitivity in *Ce-rdh-1* RNAi

To study the relationship between the hyper-resistance to X-ray irradiation of the pachytene nuclei and the activity of meiotic recombination, X-ray sensitivity was measured in *Ce-rdh-1* gene-silenced animals using an RNAi method (18). It has been reported that the effect of RNAi occurs at the post-transcriptional stage and causes degradation of specific (homologous) messenger RNA with exogenous double-stranded RNA (dsRNA) (18,23). In general, injected dsRNA can be completely transferred to eggs (F1 generation) 24 h after injection, and thus endogenous mRNA in the eggs (e.g. *mex-3* that is abundant mRNA in the gonad and early embryos) can be eliminated by the RNAi (18). Young adult hermaphrodites were injected with *Ce-rdh-1* dsRNA (injected animal: I0) and irradiated 24 h later. During this period, the strong expression of *Ce-rdh-1* mRNA in the gonads of the I0 animals was reduced to undetectable levels (Fig. 3). The hatching rate of their eggs (F1 generation) laid during 0–8 and 8–24 h after irradiation was scored (Fig. 4B). This indicates that during 8–24 h, 68.9% of eggs in the I0 animal could progress through meiosis by using the CeRDH1 protein synthesized before the dsRNA injection. All of the surviving F1 progenies during 0–24 h were morphologically normal and grew to adults, but none of their eggs that were laid (F2 generation) hatched, indicating that these F1 progenies were carriers ('escapers') with *Ce-rdh-1* dsRNA. In the 'escapers', the CeRDH1 protein level decreased sufficiently to prevent production of the F2 progeny. When the I0 animals were irradiated with 20 Gy of X-rays, the hatching rate of the egg during 0–8 h decreased to ~70% of that of the control whereas during 8–24 h, the hatching rate decreased drastically to ~10% of that of the control (Fig. 4B). Since spermatogenesis would have been completed before the dsRNA injection and irradiation, the difference in sensitivity to irradiation would be dependent on oogenesis.

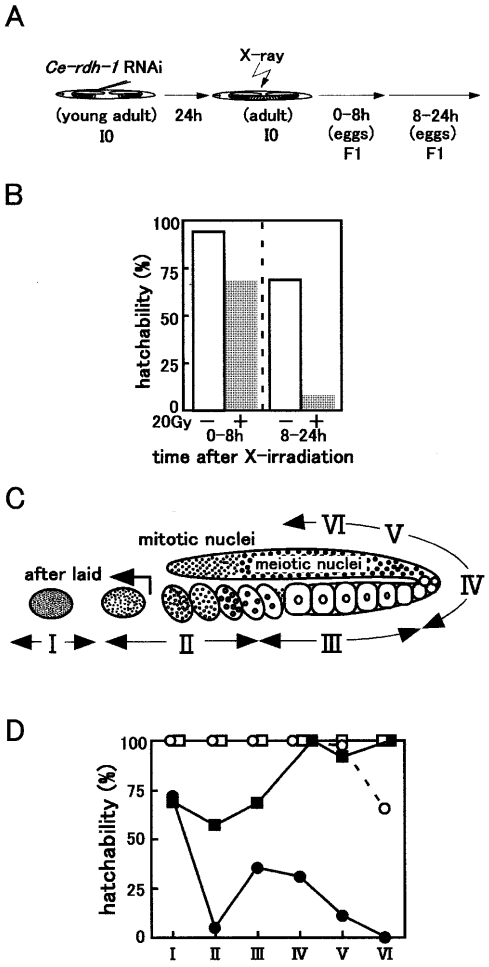

Figure 4. Effects of *Ce-rdh-1* RNAi on the X-ray sensitivity of meiotic cells and early embryos. (**A**) Schematic diagram of the experimental procedure of X-ray irradiation with *Ce-rdh-1* RNAi. (**B**) Survival of the eggs from *Ce-rdh-1* RNAi hermaphrodite following irradiation with 20 Gy of X-rays. After X-ray irradiation, the number of eggs that hatched (F1 generation) was scored during 0–8 h (average 34 eggs laid per I0 animal) and 8–24 h (average 62 eggs laid), respectively. Independent duplicate experiments were carried out and the averages of scores were plotted. (**C**) Schematic illustration of gametogenesis and early embryogenesis in a hermaphrodite. The early embryonic stage containing ~150–550 nuclei (period I: 1–4 h after laying), the early embryonic stage just after fertilization (period II: –0.5 to 1 h after laying), the fertilization stage to the full grown oocyte stage (III: –4 to –0.5 h after laying), the oocyte stage to the late pachytene stage (IV: –8 to –4 h after laying) and the pachytene nuclei stage (V: –16 to –8, VI: –24 to –16 h after laying) were used. (**D**) Survival of eggs laid during periods I–VI by *Ce-rdh-1* RNAi hermaphrodite following X-ray irradiation. Twenty-four hours after *Ce-rdh-1* RNAi, the I0 hermaphrodites were irradiated with 40 Gy of X-rays. Open squares, control with mock injection without irradiation; closed squares, X-ray irradiation with mock injection; open circles, no irradiation with the RNAi; closed circle, X-ray irradiation with the RNAi. Independent duplicate experiments were carried out and the averages of scores were plotted (average 12, 14, 22, 16, 40 and 32 eggs per animal in periods I–VI).

These results indicate that the repression of *Ce-rdh-1* expression by RNAi in meiotic pachytene cells results in the loss of hyper-resistance to X-ray irradiation. The meiotic cells become more sensitive to DSBs than the early embryonic cells (Fig. 4B). The drastic reduction of hyper-resistance to irradiation by *Ce-rdh-1* repression is probably a reflection of the essential role of this

gene product in repair of DSBs, normally a part of homologous recombination, in these meiotic cells. It has been shown that the chromosomes of *C.elegans* are holocentrically organized in mitosis, but not in meiosis (monocentrically organized) (24), suggesting the randomly end-joined chromosomes (translocation chromosomes) and fragments (called free duplications) are more stable propagated in somatic cells. Therefore, in future we have to study the activity of end-joining repair systems after X-ray irradiation with *Ce-rdh-1* RNAi in the early embryonic cells.

The results of the effect of *Ce-rdh-1* RNAi on X-ray sensitivity at a dose of 40 Gy are also shown in Figure 4D. In the control without RNAi (mock injected), the most X-ray sensitive stage was the experimental period II, –0.5 to 1 h after laying, which corresponded to the stage of early embryogenesis just after fertilization (see Fig. 4C). This was similar to the results previously described (25). After period II, the sensitivity to irradiation decreased. Repression of *Ce-rdh-1* expression with RNAi resulted in significantly increased sensitivity to irradiation in period II and prevented the decrease in sensitivity after period V (Fig. 4D).

DMC1 (*LIM15*) is essential for meiotic recombination in yeast, plant and mammals (12–15,26), and directly interacts with *RAD51* gene products in human (15). Since the repression of the nematode single *recA*-like gene *Ce-rdh-1* causes increase in radiation sensitivity of both embryos and meiotic cells (Fig. 4) and also a defect of meiotic recombination (16), the *Ce-rdh-1* may possess combined functions of *RAD51* and *DMC1* (*LIM15*) which are separately present in other eukaryotes. Rinaldo *et al.* (17) proposed a similar hypothesis in which duplication of the original eukaryotic *recA*-like gene to yield *RAD51* and *DMC1* (*LIM15*) did not occur in the evolution of *C.elegans*.

Oocyte chromosome structure after X-ray irradiation

The chromosome structure in full-grown oocytes 20 h following X-ray irradiation was studied and the result is shown in Figure 5. In the controls with mock injection, irradiated with 40 Gy of X-rays, the oocyte chromosomes appeared normal and progressed to the diakinesis stage, and six bivalents were observed. In contrast, in animals injected with *Ce-rdh-1* dsRNA and irradiated with only 20 Gy of X-rays, the condensation of the oocyte chromosomes was inhibited and kinky chromosomes were observed. This aberration of chromosomal morphology was quite similar to that observed in F1 adult progeny of parents injected with *Ce-rdh-1* RNAi without X-ray irradiation. It appears that activation of the non-homologous end joining repair system, which is normally repressed by the homologous recombination repair system, resulted in random joining of chromosomes at the DSB sites. Therefore, all of the eggs died in early embryogenesis due to their inability to complete meiotic divisions.

Gartner *et al.* (27) recently reported that the role of a checkpoint mediates DNA damage-induced apoptosis during *C.elegans* oogenesis. In contrast, *C.elegans* embryos appear to have little or no checkpoint control that respond to genotoxic stress (28). Therefore, it cannot be ruled out that the hyper-resistance of meiotic cells to ionizing radiation is an effect of the checkpoint system. However, the radiation resistant level of the meiotic cells in the *ced-3* mutant, which have a defect of apoptosis, was still sufficiently higher than that of the wild-type embryos (27,29). This suggests that meiotic cells (pachytene nuclei) are hyper-resistant to X-ray irradiation due mainly to the high levels of expression of the enzyme(s) for meiotic recombination. This phenomenon is probably ubiquitous because the meiotic process, including *recA*-like enzymes and the homologous recombination system, is widely conserved in all eukaryotes (8,21).

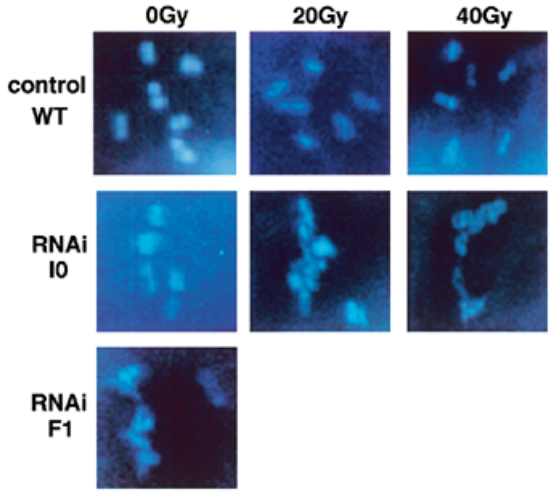

Figure 5. Aberration of oocyte chromosomes by X-ray irradiation with *Ce-rdh-1* RNAi. The chromosome structure in full-grown oocytes at diakinesis stage, ~20 h after X-ray irradiation, was examined with DAPI staining as described (16).

ACKNOWLEDGEMENTS

We thank the *C.elegans* Genome Sequencing Consortium for their great achievements, Ikuma Maeda for kindly teaching the *in situ* hybridization methods, Yoshiko Kubota and Akira Yasui for help in X-ray irradiation, Tadashi Sato for help in UV measurement, and the *C.elegans* Genetic Center for kindly providing the useful mutant strains. We are grateful to David Greenstein, Yasuo Hotta, Kensuke Horiuchi and Hideyuki Ogawa for helpful comments and discussions concerning the manuscript. This work was supported by the Joint Research Program of the Institute of Genetic Ecology, Tohoku University, Nissan Science Foundation and grants-in-aid for Priority Areas Research C (cancer: 12213015) and for Basic Research (10680646 to A.H. and 06274 to T.T.) from the Ministry of Education, Science and Culture of Japan.

REFERENCES

1. Friedberg,E.C., Walker,G.C. and Siede,W. (1995) *DNA Repair and Mutagenesis*. American Society for Microbiology Press, Washington, DC.
2. Shinohara,A. and Ogawa,T. (1995) *Trends Biochem. Sci.*, **237**, 387–391.
3. Jackson,S.P. and Jeggo,P.A. (1995) *Trends Biochem. Sci.*, **237**, 412–415.
4. Schedl,T. (1997) In Riddle,D.L., Blumenthal,T., Meyer,B.J. and Priess,J.R. (eds), *C.elegans II*. Cold Spring Harbor Laboratory Press, New York, NY, pp. 241–270.
5. Hall,D.H., Winfrey,V.P., Blaeuer,G., Hoffman,L.H., Furuta,T., Rose,K.L., Hobert,O. and Greenstein,D. (1999) *Dev. Biol.*, **212**, 101–123.
6. Hubbard,E.J.A. and Greenstein,D. (2000) *Dev. Dyn.*, **218**, 2–22.
7. Seydoux,G. and Schedl,T. (2000) In Etkin,L.D. and Jeon,K.W. (eds), *Establishment of Cell Lineages and Patterning of the Embryo*. *Int. Rev. Cytology*, Vol. 203. Academic Press, San Diego, CA, in press.

8. Roeder,G.S. (1997) *Genes Dev.*, **11**, 2600–2621.
9. Keeney,S., Giroux,C.N. and Kleckner,N. (1997) *Cell*, **88**, 375–384.
10. Dernburg,A.F., McDonald,K., Moulder,G., Barstead,R., Dresser,M. and Villeneuve,A.M. (1998) *Cell*, **94**, 387–398.
11. Shinohara,A., Ogawa,H. and Ogawa,T. (1992) *Cell*, **69**, 457–470.
12. Bishop,D.K., Park,D. Xu,L. and Kleckner,N. (1992) *Cell*, **69**, 439–456.
13. Pittman,D.L., Cobb,J., Schimenti,K.J., Wilson,L.A., Cooper,D.M. and Schimenti,J.C. (1998) *Mol. Cell*, **1**, 697–705.
14. Yoshida,K., Kondoh,G., Matsuda,Y., Habu,T., Nishimune,Y. and Morita,T. (1998) *Mol. Cell*, **1**, 707–717.
15. Masson,J.-Y., Davies,A.A., Hajibagheri,N., Dyck,E.V., Benson,F.E., Stasiak,A.Z., Stasiak,A. and West,S.C. (1999) *EMBO J.*, **18**, 6552–6560.
16. Takanami,T., Sato,S., Ishihara,T., Katsura,I., Takahashi,H. and Higashitani,A. (1998) *DNA Res.*, **5**, 373–377.
17. Rinaldo,C., Ederle,S., Rocco,V. and La Volpe,A. (1998) *Mol. Gen. Genet.*, **260**, 289–294.
18. Fire,A., Xu,S., Montgomery,M.K., Kostas,S.A., Driver,S.E. and Mello,C.C. (1998) *Nature*, **391**, 806–811.
19. Iwasaki,K., McCarter,J., Francis,R. and Schedl,T. (1996) *J. Cell Biol.*, **134**, 699–714.
20. Tabara,H., Motohashi,T. and Kohara,Y. (1996) *Nucleic Acids Res.*, **24**, 2119–2124.
21. Thacker,J. (1999) *Trends Genet.*, **15**, 166–168.
22. Beanan,M.J. and Strome,S. (1992) *Development*, **116**, 755–766.
23. Sharp,P.A. (1999) *Genes Dev.*, **13**, 139–141.
24. Albertson,D.G., Rose,A.M. and Villeneuve,A.M. (1997) In Riddle,D.L., Blumenthal,T., Meyer,B.J. and Priess,J.R. (eds), *C.elegans II.* Cold Spring Harbor Laboratory Press, New York, NY, pp. 47–78.
25. Ishii,N. and Suzuki,K. (1990) *Int. J. Radiat. Biol.*, **58**, 827–833.
26. Couteau,F., Belzile,F., Horlow,C., Grandjean,O., Vezon,D. and Doutriaux,M.P. (1999) *Plant Cell*, **11**, 1623–1634.
27. Gartner,A., Milstein,S., Ahmed,S., Hodgkin,J. and Hengartner,M.O. (2000) *Mol. Cell*, **5**, 435–443.
28. Jones,C.A. and Hartman,P.S. (1996) *Photochem. Photobiol.*, **63**, 187–192.
29. Hartman,P., Goldstein,P., Algarra,M., Hubbard,D. and Mabery,J. (1996) *Mutat. Res.*, **363**, 201–208.